

## Antagonistic effect of photosynthetic electron transport inhibitors on peroxidizing phytotoxic activity

H Kohno,<sup>1\*</sup> A Ohki,<sup>1</sup> K Koizumi,<sup>2</sup> N Kuboyama,<sup>1</sup>  
S Ohki,<sup>1</sup> P Böger<sup>3</sup> and K Wakabayashi<sup>1</sup>

<sup>1</sup>Graduate School of Agricultural Science, Tamagawa University,  
Machida-shi, Tokyo 194, Japan

<sup>2</sup>Research Laboratory, Tomono Agric Co Ltd, 290 Ohyanagi,  
Shimada-shi, Shizuoka-ken 427-01, Japan

<sup>3</sup>Lehrstuhl für Physiologie und Biochemie der Pflanzen,  
Universität Konstanz, D-78434 Konstanz, Germany

**Abstract:** It is shown that a number of new 2-benzylamino-1,3,5-triazines have a similar antagonistic effect to diuron on the phytotoxic activity of peroxidizing herbicides.

**Keywords:** peroxidizing herbicides; photosynthetic electron transport inhibitors; diuron effect

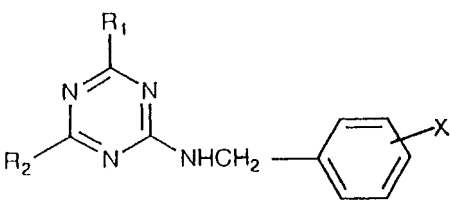
The first effect of peroxidizing herbicides is to inhibit protoporphyrinogen-IX oxidase. This inhibition is accompanied by an abnormal accumulation of protoporphyrin-IX. The accumulated protoporphyrin-IX acts a photosensitizer in the light, induces active oxygen species, and subsequently degrades cellular constituents. It is known that diuron alleviates the action of peroxidizing herbicides in autotrophic *Scenedesmus acutus* cells.<sup>1</sup> Chlorophyll decrease, short-chain hydrocarbon formation and protoporphyrin-IX (Proto-IX) accumulation in cells caused by peroxidizing herbicides are counteracted by simultaneous addition of diuron.<sup>2</sup> This alleviation of peroxidation by photosynthetic electron transport (PET) inhibitors (called the 'diuron effect') is an antagonistic effect observed with autotrophic cells.<sup>3</sup> In this summary, we report results of assays of several 2-benzylamino-1,3,5-triazines<sup>4</sup> and diuron as PET inhibitors (Table 1).

PET inhibitory activities ( $pI_{50}$ ) of 2-benzylamino-1,3,5-triazines were determined with spinach thylakoids, before starting the experiment to check the antagonistic effects of these compounds on phytotoxic activity caused by peroxidizing herbicides.

All 2-benzylamino-1,3,5-triazines inhibited photosynthetic oxygen evolution by spinach thylakoids. The order of PET inhibitory activities was  $8 > 3 > 6 > 4 \approx 5 > 7 \approx 2 > 1$ . The extent of inhibition with these compounds was of the same order as that of the reference PET inhibitor, diuron.

Effects of 2-benzylamino-1,3,5-triazines on the phytotoxic activity of oxyfluorfen were examined with *Scenedesmus* cells. In light-cultured *Scenedesmus* cells neither ethane formation nor Proto-IX accumulation was observed with 2-benzylamino-1,3,5-triazines or diuron without a peroxidizer. A chlorophyll decrease

**Table 1.** Inhibitory effects of 2-benzylamino-1,3,5-triazines on spinach thylakoids



Compound	R <sub>1</sub>	R <sub>2</sub>	X	$pI_{50}$ <sup>a</sup>
1	H	CH <sub>3</sub>	—	<4.00
2	CH <sub>3</sub>	CH <sub>3</sub>	—	4.19
3	CH <sub>3</sub>	CF <sub>3</sub>	—	6.85
4	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	—	5.16
5	CH <sub>3</sub>	C <sub>3</sub> H <sub>7</sub> -iso	—	5.13
6	CF <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	—	5.64
7	CF <sub>3</sub>	C <sub>3</sub> H <sub>7</sub> -iso	—	4.21
8	CF <sub>3</sub>	CH <sub>3</sub>	4-Br	6.94
Diuron				6.93

<sup>a</sup>  $pI_{50}$  (spinach thylakoids) represents inhibition of the Hill reaction by the compounds tested (system  $H_2O \rightarrow$  ferri-cyanide, uncoupled with  $NH_4Cl$ ).

was not observed with  $10^{-5}$  M triazine (Table 2). Thus, it is evident that PET inhibitors themselves do not act as peroxidizers.

The chlorophyll decrease caused by oxyfluorfen was alleviated by simultaneous addition of 2-benzylamino-1,3,5-triazines to light-cultured *Scenedesmus* cells. The light-induced ethane formation caused by oxyfluorfen was almost abolished by compound 8 or diuron, when added simultaneously but was not alleviated by compounds 1 and 2 (Table 3). Proto-IX accumulation was also counteracted with compounds 3 and 8, and with diuron.

Apparently the degrees of alleviation of chlorophyll decrease and ethane formation were proportional to the PET inhibitory activity of the compounds. Ethane formation and chlorophyll decrease caused by oxyfluorfen indicate a good correlation with PET inhibi-

**Table 2.** Phytotoxic activities of 2-benzylamino-1,3,5-triazines ( $10^{-5}$  M) in the light in autotrophic *Scenedesmus acutus* cells after 16h incubation

Compound	Growth ( $\mu l$ pcv $ml^{-1}$ ) <sup>a</sup>	Chlorophyll content	
		( $\mu g$ $ml^{-1}$ )	( $mg$ $ml^{-1}$ pcv)
At start	2.4	24.66	10.28
Control	3.6	26.18	7.27
1	3.2	24.94	7.79
2	3.6	27.19	7.55
3	3.4	28.12	8.27
4	3.6	33.21	9.23
5	2.8	35.43	12.65
6	3.2	29.51	9.22
7	3.6	29.82	8.28
8	3.4	25.90	7.62
Diuron	3.4	26.88	7.90

<sup>a</sup> pcv = packed cell volume.

\* Correspondence to: H Kohno, Graduate School of Agricultural Science, Tamagawa University, Machida-shi, Tokyo 194, Japan (Received 29 June 1998; accepted 1 February 1999)

Culture condition	Growth ( $\mu\text{l pcv ml}^{-1}$ )	Chlorophyll content		Ethane formation ( $\text{nmol ml}^{-1} \text{pcv}$ )	Proto-IX accumulation <sup>a</sup> ( $\text{nmol ml}^{-1} \text{pcv}$ )
		( $\mu\text{g ml}^{-1}$ )	( $\text{mg ml}^{-1} \text{pcv}$ )		
At start	2.4	27.40	11.42	—	—
Control	3.6	33.3	8.98	nd <sup>b</sup>	2.45
Oxyfluorfen	3.4	5.30	1.56	13.38	106.43
(+) <b>1</b>	3.2	5.43	1.70	12.19	
(+) <b>2</b>	3.4	6.09	1.79	12.29	
(+) <b>3</b>	3.4	17.61	5.18	3.82	42.52
(+) <b>4</b>	3.6	12.18	3.38	11.00	
(+) <b>5</b>	3.2	8.30	2.59	10.83	
(+) <b>6</b>	3.2	16.18	5.06	10.00	
(+) <b>7</b>	3.4	6.22	1.83	11.47	
(+) <b>8</b>	3.2	26.85	7.46	n.d.	30.21
(+) Diuron	3.6	25.75	7.15	n.d.	32.44

**Table 3.** Effect of 2-benzylamino-1,3,5-triazines ( $10^{-5}\text{M}$ ) on the peroxidizing activity of oxyfluorfen in *Scenedesmus acutus* cells after 16h incubation in the light

<sup>a</sup> Proto-IX accumulation after a 2-h incubation.

<sup>b</sup> nd = not detected.

tory activity ( $\text{pI}_{50}$ ) of compounds, as shown by the following equations:

$$\text{Ethane formation} = -3.834 \text{ pI}_{50}(\text{thylakoids}) + 29.366 (\pm 1.902)$$

$$[n = 7, r = 0.92, s = 2.03]$$

$$\text{Chlorophyll content} = 1.742 \text{ pI}_{50}(\text{thylakoids}) - 5.587 (\pm 0.789)$$

$$[n = 7, r = 0.93, s = 0.84]$$

## REFERENCES

- Kunert KJ and Böger P, *Weed Sci* **29**:169–173 (1981).
- Böger P and Wakabayashi K, *Z Naturforsch* **50c**:159–166 (1995).
- Ohki A, Ohki S, Koizumi K, Sato Y, Kohno H, Böger P and Wakabayashi K, *Nihon Noyaku Gakkaishi (J Pestic Sci)* **22**:309–313 (1997).
- Koizumi K, Kuboyama N, Tomono K, Tanaka A, Ohki A, Kohno H, Böger P and Wakabayashi K, *Pestic Sci* **55**:645–646 (1999).

## Steric structure–activity relationships of the rice blasticide, S-2900 (diclocymet)

Akio Manabe, Masayuki Enomoto, Yoshimi Yamada, Yukio Oguri and Mitsuru Sasaki\*  
Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co. Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665855, Japan

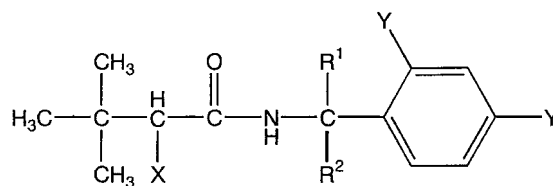
**Abstract:** The four diastereomers of 2-cyano-*N*-[1-(2,4-dichlorophenyl)ethyl]-3,3-dimethyl-butyr-*amide* were prepared by a direct HPLC separation with chiral columns. The [(*S*)acid, (*R*)amine]-isomer (was the most antifungal among the diastereomers tested. Because of the lability of the clinical group in the acid moiety, the (*RS*)-(*R*)-isomer is

being developed as a rice blasticide. (**S-2900**, proposed common name diclocymet).

**Keywords:** S-2900; rice blasticide; optically active diastereomer

During the course of a study on modifications to the structure of the herbicide bromobutide (Fig 1; **1**),<sup>1</sup> we found that its cyanobutyramide derivatives are effective in controlling rice blast disease caused by *Pyricularia oryzae* Bri & Cavara. Of these, 2-cyano-*N*-[1-(2,4-dichlorophenyl)ethyl]-3,3-dimethylbutyr-*amide* (**2**) was selected as a potential new rice blasticide. The molecule has two stereogenic centres, one in the acid and one in the amine moiety. To examine the steric structure/biological activity relationships of the molecule, the four possible stereoisomers were prepared by optical resolution of 1-(2,4-dichlorophenyl)ethylamine followed by column chromatographic separation or by recrystallization of the optically active diastereomeric mixture of the cyanobutyramide. In particular, the racemic diastereomers could be separated cleanly using HPLC with chiral columns such as Sumipax YMC-Gel and Sumichiral OA-4700. The absolute configuration of one of the diastereomers, mp: 180–186 °C,  $[\alpha]_{\text{D}} = +45.9^\circ$ , was determined to be the (*R,R*)-configuration by X-ray single crystal analysis.

As shown in Table 1, the diastereomers with an (*R*)-configuration at the amine moiety exhibited considerable antifungal activity, while those with an (*S*)-



**Figure 1.** Structures of compounds discussed. Bromobutide **1**: X = Br, Y = H, R<sup>1</sup> = R<sup>2</sup> = CH<sub>3</sub>; **2**: X = CN, Y = Cl, R<sup>1</sup> = CH<sub>3</sub>, R<sup>2</sup> = H.

\* Correspondence to: Mitsuru Sasaki, Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co. Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665855, Japan  
E-mail: sasakim5@sc.sumitomo-chem.co.jp  
(Received 23 June 1998; accepted 1 February 1999)